Research Article

Insights into the ecological impact of trout introduction in an oligotrophic lake using sedimentary environmental DNA

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Abstract

Introduced trout can induce trophic cascades, however, a lack of pre-introduction data limits knowledge on their impact in many lakes. Traditional paleolimnological approaches have been used to study historic species changes, but until recently these have been restricted to taxa with preservable body-parts. To explore the ecosystem effects of *Salmo trutta* (brown trout) introduction on an oligotrophic lake in Aotearoa-New Zealand, we used a multi-marker sedimentary environmental DNA (sedDNA) approach coupled with pigments to detect changes across multiple trophic levels. DNA was extracted from core depths capturing approximately 100 years before and after the expected arrival of *S. trutta*, and metabarcoding was undertaken with four primer sets targeting the 12S rRNA (fish), 18S rRNA (eukaryotes) and cytochrome c oxidase (COI; eukaryotes) genes. The earliest detection of *S. trutta* eDNA was 1906 (1892–1919 CE with 95% high probability density function) suggesting their introduction was shortly before this. Native fish diversity (12S and 18S rRNA) decreased after the detection of *S. trutta*, albeit the data was patchy. A shift in overall eukaryotic and algal communities (18S rRNA and COI) was observed around 1856 (1841–1871 CE) to 1891 (1877–1904 CE), which aligns with the expected *S. trutta* introduction. However, taxonomy could not be assigned to many of the 18S rRNA and COI sequences. Pigment concentrations did not change markedly after *S. trutta* introduction. SedDNA provides a new tool for understanding the impact of disturbances such as the introduction of non-native species; however, there are still several methodological challenges to overcome.

Key words: brown trout, food web, multi-marker, sedDNA, sediment core, 12S rRNA, 18S rRNA and cytochrome c oxidase

Introduction

Globally, the health of lakes is being threatened by multiple stressors including increased nutrient and sediment inputs, climate change and the introduction of non-native species (Hecky et al. 2010; Wang et al. 2021). Intentional introductions of non-native species have been undertaken to enhance the amenity values of lakes, for example, the widespread release of *Micropterus salmoides* (largemouth bass) across North America due to its popularity as a sport fish (Brown et al. 2009). Alternatively, non-native species can be introduced to lakes unintentionally, for example goldfish (Collier and Grainger 2015) as well as other taxa such as the diatom *Lindavia intermedia* (Kilroy et al. 2021; Schallenberg et al. 2022) and macrophytes including *Egeria* and *Myriophyllum* (Hussner et al. 2010; Gillard et al. 2017).

While not all introduced fish establish successfully, those that do can have wide-reaching direct and indirect effects on lake ecosystems (Cucherousset and Olden 2011). These impacts include competition for resources or habitat, predation, the induction of genetic and behavioural shifts in local species, changes in foodwebs and sometimes localised extinctions (Gozlan et al. 2010; Cucherousset and Olden 2011; Milardi et al. 2016). In some areas, non-native fish have established themselves with such success that they outnumber native fish, for example, a study of 200 lakes in north-eastern USA showed greater densities of non-native compared to native fish in over one third of the study lakes (Whittier and Kincaid 1999).

A key challenge in understanding the impacts of non-native fish on lake ecosystems is the requirement of pre-introduction data on the abundance and composition of lake foodwebs. However, most lake monitoring records are temporally limited and do not include data pre-introduction, or if it is available, this data is usually only related to water quality and physicochemical variables. The lack of pre-introduction data is particularly troublesome in countries like Aotearoa-New Zealand where acclimatisation societies facilitated the shipment and widespread release of animals and plants from Britain during the early periods of European colonization from about 1860 CE onwards (Walrond 2008). Prior to this, lacustrine native fish communities consisted mainly of generalist feeders including *Galaxiid*, *Gobiidae*, *Anguillid* and *Retropinna* species (Kelly and McDowall 2004). The apex fish predators were likely *Anguilla* (eels) and some of the larger *Galaxias* species (i.e., kōkopu) that are effective generalists, large enough to predate many of the smaller native fish species (e.g., Jellyman 1996; Stewart et al. 2022). Due to the more generalist feeding habits of many native fish in Aotearoa-New Zealand, lake food webs lacked obligate piscivorous, zooplanktivorous and herbivorous fish (Burns 1998). The resulting foodwebs were relatively simple, lacking the strict trophic boundaries seen in many North American temperate lakes which can lead to cascading foodweb effects (Rowe and Schallenberg 2004). As no pre-introduction data exists in these systems, the effect of trout introduction on these foodwebs is unknown. In addition, with the exception of remote lakes in National Parks, alpine lakes or those that lack connectivity to tributaries, most lakes in Aotearoa-New Zealand now contain non-native fish (e.g. https://niwa.co.nz/freshwater/nzffd/NIWA-fish-atlas for species distribution maps). This makes

comparative contemporary studies between lakes with and without fish introductions near impossible.

The widespread introduction of non-native fish species such as rainbow (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) to Aotearoa-New Zealand rivers in the late 1800's CE was so successful that *S. trutta* are now the most widespread and often most abundant fish species in Aotearoa-New Zealand waterbodies (Jones and Closs 2017). The presence of *S. trutta* has been shown to negatively impact native invertebrate and fish communities in streams in Aotearoa-New Zealand (Townsend and Simon 2006), where they feed on a wide variety of prey including aquatic and terrestrial arthropods and molluscs (Rutledge 1991; Sagar and Glova 1995). However, Wissinger et al. (2006) found no effect of *S. trutta* on benthic invertebrates in small lakes in Aotearoa-New Zealand, contrasting with studies from Northern Hemisphere lakes with salmonid introductions (Luecke 1990; Carlisle and Hawkins 1998). Due to a lack of pre-introduction native fish data and the relatively simple pre-existing foodwebs in lakes in Aotearoa-New Zealand, the effects of *S. trutta* introduction on these foodwebs remain poorly understood.

In order to understand the impacts of non-native species in the absence of pre-introduction data, paleolimnological investigations have been conducted using fossil assemblages (e.g., Sweetman and Smol 2006; Palm and Svensson 2010). Changes in zooplankton and diatom abundance, community structure and zooplankton size distributions have been correlated with trout introduction using these methods (Palm and Svensson 2010; Cantonati et al. 2021). However, these techniques can only target organisms that leave preservable fossil remains in the sediment. The advent of molecular techniques that target sedimentary environmental DNA (sedDNA) offers the potential to recover a wide variety of organism signals in lake sediments, as DNA is shed and excreted by all organisms (Capo et al. 2021; Barouillet et al. 2022). The application of sedDNA to obtain pre-introduction baseline data across a broad array of taxa therefore has the potential to enable new insights into the wider impacts of non-native fish introductions in lakes.

The aim of this study was to apply sedDNA techniques to samples from a lake sediment core covering periods before and after the likely introduction of *S. trutta* and explore responses across a range of organisms. To achieve this, we used a multi-marker sedDNA metabarcoding approach on a sediment core retrieved from Lake Paringa, a lake situated in a relatively unaltered native forest catchment on the West Coast of Aotearoa-New Zealand with a previously described core chronology and 14C dating. *S. trutta* are likely to have been released into this area around 1870 CE (Jones and Closs 2017). We focused on regions of the 18S ribosomal RNA (rRNA) targeting eukaryotes, 12S rRNA targeting fish, and two regions of the cytochrome c oxidase subunit 1 mitochondrial gene (COI) targeting eukaryotes, in particular macroinvertebrates, over a time period spanning approximately 100 years before and after *S. trutta* introduction. These data were initially investigated to identify when trout were introduced to Lake Paringa as no written records could be located. The data was then explored to assess community wide changes. Algal pigments were analysed using high performance liquid chromatography (HPLC) to provide quantitative data on shifts in algal abundance.

Methods

Study lake

Lake Paringa is a moderately sized (max. depth 58 m, area 460 ha), oligotrophic lake located on the West Coast of the South Island of Aotearoa-New Zealand (43°43'10"S, 169°24'8"E; Fig. 1). The catchment (64 km^2) is mainly temperate rainforest which has been largely undisturbed by human activities (Howarth et al. 2012). The West Coast is the wettest region in the country, due to the predominant westerly airflow and orographic effect of the Southern Alps. The area around Lake Paringa receives on average between 4,000–6,000 mm of rain per year (Macara 2016). The lake is culturally significant for Māori (indigenous people of Aotearoa-New Zealand). Seasonal kāinga nohoanga (small settlements) were established near Lake Paringa around 1300 CE, and the lake provided an abundant supply of food. Lake Paringa was, and still is, a noted tuna (eel) fishery, significant spawning ground and nursery for a variety of fish species, and a significant breeding area for birds (Ngāi Tahu Claims Settlement Act 1998). European access to this lake likely began around 1875 with the construction of the Haast to Paringa Cattle Track which allowed famers to move cattle along this rugged region of the West Coast. The track originally passed directly along the Eastern side of the lake following the route of the current main highway (Fig. 1). In 1965, the Haast Pass Highway was constructed, opening up this region of the West Coast for motorised travel from Central Otago to the West Coast.

The sedimentology of Lake Paringa has been well characterised by previous studies (Howarth et al. 2012, 2021). Sediment cores from the lake are characterised by four lithofacies that form a repeating sequence related to the earthquake cycle of the Alpine Fault. The sequence includes: (i) rapidly deposited layers inferred to be co-seismic debrites and overlying turbidites formed by shaking-induced subaqueous mass wasting; (ii) stacks of rapidly deposited layers interpreted as hyperpycnites formed during periods of elevated fluvial sediment flux in response to earthquake-induced land sliding; and (iii) layered silts formed between earthquakes when the catchment was relatively geomorphically quiescent. In the present study we targeted layered silts that have accumulated post 1800 CE which are uninfluenced by deposition associated with the Alpine Fault seismic cycle.

Sample collection and subsampling

A 6-meter-long sediment core was taken in the deepest point of the Windbag basin of Lake Paringa (58 m; Fig. 1) using a Mackereth corer (Mackereth 1958). The sediment core was transported chilled in a refrigerated trailer (4 °C) to the laboratory and stored (4 °C) for 30 days before being frozen (-20 °C). In the laboratory, the core was thawed and split in half using an electric saw to cut the plastic core tube and a sterile metal blade was used to slice the sediment horizontally across the layers to prevent vertical contamination. The core halves were photographed using a camera (Suppl. material 1: fig. S1) and logged in detail. Subsamples of sediments for DNA analysis (*n* = 42; Suppl. material 1: table S1) were taken from half of the core. By targeting layered silts that were deposited from more autochthonous sources, we optimised the subsampling to target sedDNA from aquatic organisms.

The top 2–3 mm of the sediment core surface were scraped off using sterile spatulas to remove potentially contaminated sediment from the splitting
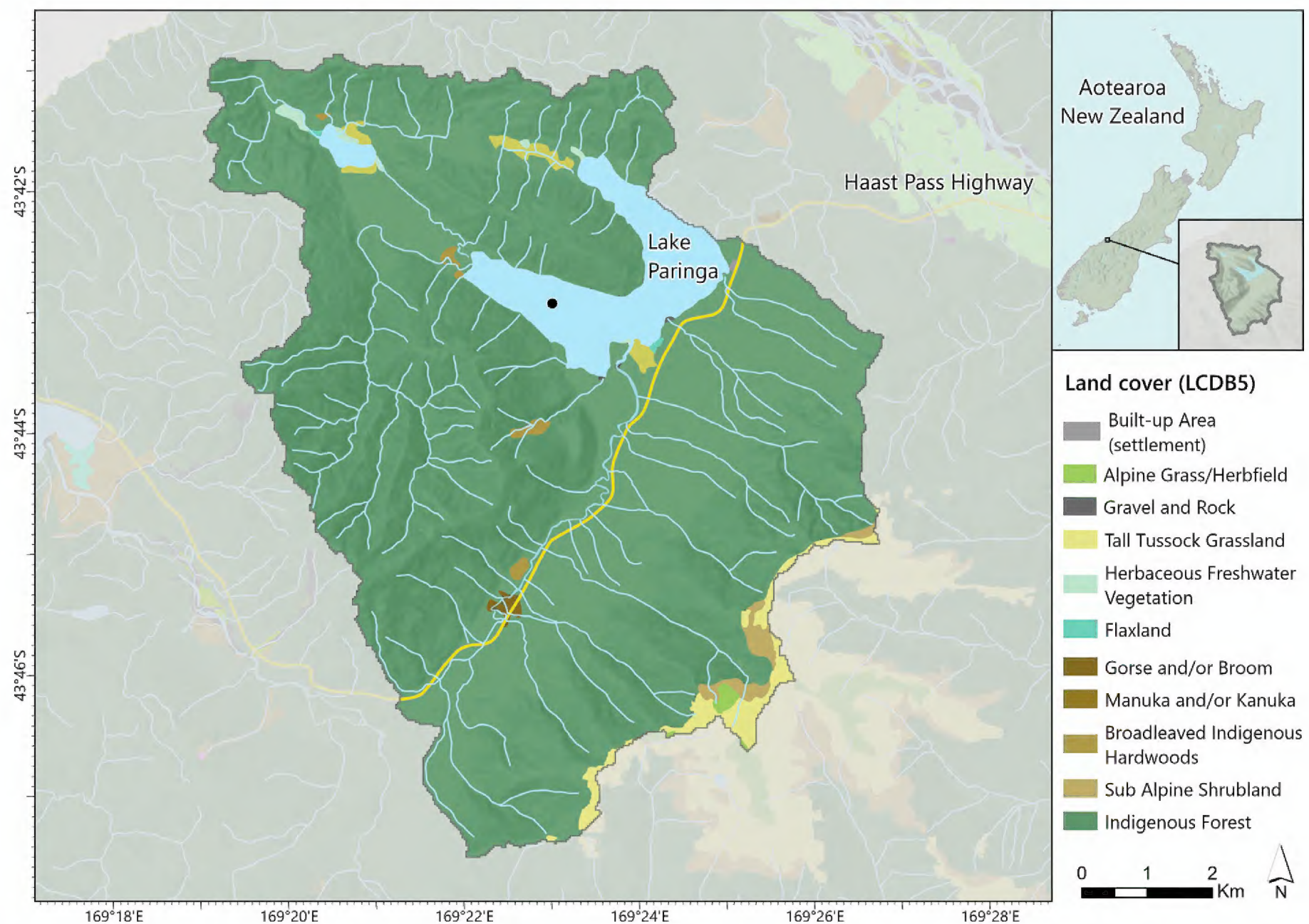
Figure 1. The Location of Lake Paringa on the West Coast of Aotearoa-New Zealand. The black dot represents the core location. Colours represent catchment land cover classes outlined in the legend. The yellow line shows the location of the Haast Pass Highway, parts of which were once the Haast to Paringa Cattle Track. Light blue lines represent streams and rivers within the catchment. LCDB5 = land cover database version 5 - https://lris.scinfo.org.nz/layer/48423-lcdb-v41-deprecated-land-cover-database-version-41-mainland-new-zealand/.

process. Samples for sedDNA analysis were collected by sampling the inner sediments (ranging from 4.5–9.5 g) using sterile spatulas. Subsamples were placed in 50-mL tubes and frozen (-20 °C) until DNA extraction.

Chronology

Detailed visual logs and linescan imagery of the sediment core were used to correlate the new core with the original core from Howarth et al. (2012) at sub-centimetre scale using diagnostic horizons that allow sub-centimetre-scale correlations (Frith et al. 2018; Wang et al. 2020). The original master-core chronology was based on accelerator mass spectrometry measurements of the radiocarbon (14C) content of 22 terrestrial macrofossils (Howarth et al. 2012). Howarth et al. (2012) derived a calibrated calendar age for each macrofossil in OxCal 4.1 using the P_sequence depositional model.

Sediment DNA extraction and sequencing

DNA was extracted from all sediment sub-samples (*n* = 42) using the Lakes ABPS extraction protocol as described in Thomson-Laing et al. (2022). Briefly, sediment samples (~3–5 g) were mixed with sodium hydroxide (6 mL, 0.33M)

and Tris-EDTA (3 mL, pH 8), vortexed (1 min), incubated at 65 °C for 50 min and centrifuged (15,000 × *g*, 1 hr, 15 °C). The resultant supernatant (7.5 mL) was transferred to a new tube and neutralized with 7.5 mL of Tris HCl (1M, pH 6.7). Sodium acetate (1.5 mL, 3M, pH 5.2) and molecular grade 100% ethanol (30 mL) were added, and samples were frozen (-20 °C) overnight to precipitate DNA. Samples were centrifuged (10,000 × *g*, 1 hr, 15 °C) and the supernatant discarded. DNA was extracted from the entire pellet using multiple DNeasy PowerSoil DNA Isolation Kits (Qiagen, Germany; one per 0.5 g pellet) and the automated Qiacube, following manufacturer's protocol. Final elution volume per extracted sample was 100 μL. Eluted DNA from the same pellet extraction were pooled together following extraction. Two negative extraction controls, where no sediment was added to the extraction tube, were included every 23rd extraction.

PCR was undertaken using primers targeting the mitochondrial 12S rRNA gene (~100 base pairs [bp]) for fish, the 18S rRNA gene (~440 bp) targeting all eukaryotes, and two regions of the mitochondrial cytochrome c oxidase I (COI) gene (~178 bp [short COI] and ~316 bp [long COI]) targeting all eukaryotes. All primers included Illumina™ adapter tails for dual-index sequencing (Kozich et al. 2013). The 18S rRNA and 12S rRNA primers are previously published, whereas the long COI forward and short COI reverse primers were modified slightly for better recovery of zooplankton species found in Aotearoa-New Zealand (Table 1). A simple *in-silico* analysis was undertaken using 99 freshwater zooplankton species found in Aotearoa-New Zealand (14 cladocera, 35 copepod and 50 rotifer species) retrieved from the BOLD database (Ratnasingham and Hebert 2007). Alignment of zooplankton sequences alongside the freshwater macroinvertebrate long COI forward primer (Elbrecht and Leese 2017) and short COI reverse primer (Vamos et al. 2017) suggested the adapted primers allowed for 34 additional bp matches, resulting in only 15 bp mis-matches (EukCOI-L, BF1-adapted) and 42 additional bp matches, resulting in only 11 bp mis-matches (EUKCOI-S, fwhR1-adapted) using the newly adapted primers.

PCR reactions (25 μL) contained 12.5 μL MyFi 2 × PCR mastermix (Bioline Reagents, London, UK), 2–5 μL of template DNA and nuclease-free water, 1 μg bovine serum albumin (UltraPure BSA, Invitrogen, Massachusetts, United States) and 450 nM of forward and reverse primer. For all PCRs, temperature cycling conditions involved an initial denaturation step of 95 °C (5 min), then assay-specific cycles of denaturation, annealing and extension

Table 1. Primers used in this study, including two cytochrome c oxidase subunit 1 mitochondrial gene (COI) primers adapted for this study. Bases modified for this study are indicated in bold.

Datasets	Gene	Primer name	Sequence	Sequence length (bp)	Target organisms	Reference
Euk18S	18S rRNA	Uni18SF	AGGGCAAKYCTGGTGCCAGC	~ 440	Eukaryotes	Zhan et al. 2013
		Uni18SR	GRCGGTATCTRATCGYCTT			
Fish12S	12S rRNA	12SV5-F	TTAGATACCCCACTATGC	~ 100	Fish / (mammals, birds)	Riaz et al. 2011
		12SV5-R1	TAGAACAGGCTCCTCTAG			
EukCOI-L and AlgaeCOI-L	CO1	BF1_adapted	AC**D**GG**D**TGRAC**H**GTNTAYCC	~ 316	Eukaryotes (macroinvertebrates plus others)	This study
		BR2	TCDGGRTGNCCRAARAAYCA			Elbrecht and Leese 2017
EukCOI-S and AlgaeCOI-S	CO1	Fwhf1	YTCHACWAAYCAYAARGAYATYGG	~ 178	Eukaryotes (macroinvertebrates plus others)	Vamos et al. 2017
		Fwhr1_adapted	AR**Y**CARTT**H**CCRAAHCCHCC			This study

(Suppl. material 1: table S2) finishing with a final extension step at 72 °C (7 min). A positive control (DNA from *Anguilla australis* for the 12S rRNA primers and from *Sabella spallanzanii* for the 18S rRNA and COI primers) and a negative template control (nuclease-free water) were included in each PCR run.

To confirm successful amplification, PCR products were visualized on a 1.5% agarose gel with Nucleic Acid Staining Solution (iNtROM Biotechnology, South Korea). PCR products (20 µL) including negative extraction and PCR controls were cleaned and normalized (to ~1 ng µL⁻¹) with SequelPrep Normalisation Plates (Applied Biosystems, CA, USA). Cleaned PCR products were then sent to Azenta (Suzhou, China) or Sequench Ltd. (Nelson, New Zealand) for sequencing (paired-end) on an Illumina Miseq™ with the V2 2×250 bp cycle kit for the 18S rRNA and COI amplicons and the V2 2×150 bp cycle kit for 12S rRNA fish amplicons. A bio-analyzer and Qubit 4 fluorometer (Thermo Fisher Scientific, Massachusetts, USA) were used to quantify the concentration and quality of the pooled library before it was denatured and diluted to a loading concentration of 6 pM with a 15% PhiX spike. The raw sequences were deposited into the NCBI short read archive (12S rRNA: PRJNA1007422; COI: PRJNA1007424; 18S rRNA: PRJNA1007437).

Bioinformatics

Processing of sequences for all genes was undertaken in the same way unless otherwise stated and scripts can be found at https://github.com/jkpearmanbio-inf/sedimentaryDNA-trout. Samples were automatically demultiplexed on the MiSeq machine based on the dual indexing. Primers were removed from the resulting sequences using Cutadapt (Martin 2011), allowing for a single mismatch. Construction of Amplicon Sequence Variants (ASVs) was undertaken in the DADA2 package v1.26 (Callahan et al. 2016) within R version 6.2 (R Core Team 2021). Forward reads were trimmed to a length of 120 bp, 230 bp, 130 bp and 165 bp for the 12S rRNA, 18S rRNA, fwhR-amplified COI and BF1-amplified COI genes, respectively, while the reverse reads were trimmed to 120 bp, 228 bp, 130 bp and 165 bp for the 12S rRNA, 18S rRNA, fwhR-amplified COI and BF1-amplified COI genes, respectively. Sequences for each gene were denoised by a pseudo-pooling approach using an error profile constructed from a parametric model derived from the first 1×10^8 bp of the sequences. ASVs were constructed by merging forward and reverse reads allowing for one mismatch and having a minimum overlap of 10 bp. Chimeric sequences were removed using the removeBimeraDenovo script within the DADA2 package. ASV tables are included as Suppl. material 2: tables S3−S11. Taxonomy was assigned in a two-stage process. Firstly, ASVs were classified using the RDP Naïve Bayesian Classifier (Wang et al. 2007) within DADA2 with a bootstrap of 70 for the 12S rRNA and 18S rRNA and 50 for the COI to enable classifications at higher taxonomic levels. The PR² (Guillou et al. 2013) database was used for the classification of 18S rRNA ASVs while databases were constructed based on NCBI (Agarwala et al. 2018) for 12S rRNA and BOLD (Ratnasingham and Hebert 2007) for COI made using CRABS v0.1.7 (Jeunen et al 2023). Secondly, species level classifications for the 12S rRNA dataset were determined using blastn (> 99% similarity for species classification) against the non-redundant NCBI nucleotide database. The ASVs, taxonomy and accompanying metadata were combined into 'phyloseq' v1.44 (McMurdie and Holmes 2013) objects which were the basis for statistical analysis.

Pigments

Core sub-samples for pigment analysis (*n* = 20) were thawed at 4 °C, weighed in Falcon tubes and extracted three-times using acetone and a bath sonicator containing ice (30 min). The extract was dried under a stream of nitrogen gas at 40 °C and stored at -20 °C and protected from the light until analysis (< 1 week). The dried extract was re-suspended in acetone on the day of analysis and transferred to a septum-capped amber vial. Extracts were analysed by HPLC with diode array detection (DAD) using an Agilent 1260 HPLC-DAD system (Santa Clara, CA, USA) as described in Short et al. (2022) but using a shorter gradient. Extract components (10 µL injection) were loaded on to a Develosil RP Aqueous column (Nomura Chemicals; 250×4.6 mm; 5-µm) at 10%B where the solvent composition was held for 2 min before proceeding in a linear gradient to 40%B over 28 min. The column was flushed with 80%B for 3 min and re-equilibrated with 10%B for 5 min. The results from all pigments analyzed (alloxanthin, canthaxanthin, diatoxanthin, lutein, myxoxanthophyll and zeaxanthin) were summed to provide an overview of changes in total algal biomass. This approach was used, rather than using chlorophyll-a, as most of the organic matter in Lake Paringa is terrestrial (Frith et al. 2018), which makes it likely that most of the chlorophyll-a is also terrestrial and therefore not necessarily the best proxy for autogenic productivity. Mass accumulation through the core was derived from sediment bulk density and water content, where bulk density was calculated from CT densitometry using the relationship of Reilly et al. (2017). The pigment data was converted to a flux using CT-derived mass accumulation rates to account for changes in sedimentation rates through time. The pigment data was assigned as pre and post *S. trutta* introduction using the date established from sedDNA. To determine if there was a significant difference before and after the arrival of *S. trutta*, a t-test was performed after assessing variance equality on the total pigment concentration and the total pigment flux data.

Statistics

All statistical analyses were performed in R (R Core Team 2021). Sequencing reads were pre-processed as follows. Sequences found in any control sample for each gene were subtracted from the datasets. Taxa with less than five total reads per dataset were removed. Sequencing datasets were rarefied to a suitable sequence depth for each gene after considering rarefaction curves (Suppl. material 1: fig. S2). The 12S rRNA gene reads were rarefied to 5,000 reads and then subset to remove birds, mammals and other taxa keeping only the Actinopteri class focusing only on fish (Fish12S rRNA). Terrestrial plant (terrestrial Streptophyta) reads were removed from the 18S rRNA gene as they were not relevant to this study, before the remaining 18S rRNA gene reads were rarefied to 20,000 reads. This gene was then subset into three groups: total eukaryotes (Euk18S), from which Chordate reads were removed, resulting in eukaryotes without fish; a subset of only fish (Fish18S), where reads were subset to include only the Actinopteri class; and groups heavily predated by trout (Prey18S) in which reads were subset to include Arthropoda, Mollusca and Rotifera. Diet studies have shown that *S. trutta* predate on both aquatic and terrestrial arthropods (Rutledge 1991; Sagar and Glova 1995), therefore

arthropods from both habitats were included in this group. The short COI gene sequences (EukCOI-S) were rarefied to 25,000 reads and bacteria, land plants (terrestrial Streptophyta), Chordates and terrestrial worms (Megascolecidae) removed. EukCOI-S was then subset into both trout diet (Phyla: Arthropoda, Mollusca and Rotifera; PreyCOI-S) and Algae (AlgaeCOI-S). The long COI gene sequences (EukCOI-L) were rarefied to 4,000 reads and subset in the same way as the EukCOI-S, resulting in PreyCOI-L and AlgaeCOI-L.

Stratigraphic cluster analysis

Downcore stratigraphic plots were created for each taxonomic group analysed from the different genes (Fish12S, Fish18S, Euk18S, EukCOI-S, EukCOI-L, Prey18S, PreyCOI-S, PreyCOI-L, Algae18S, AlgaeCOI-S and AlgaeCOI-L) using the strat.plot function in the 'rioja' package (Juggins 2022). Constrained hierarchical clustering analysis was used to determine taxa assemblage zones downcore, constrained by sampling order to determine community shifts leading to different assemblages. A Bray-Curtis dissimilarity matrix was calculated on the plotted data using the 'vegan' package v2.6-4 (Oksanen et al. 2020). Hierarchical clustering was then performed using the Constrained Incremental Sum of Squares (CONISS) approach (Grimm 1987) using the 'chclust' function in the 'rioja' package (v1.0-5). The primary major assemblage shift was determined; however, if these shifts occurred post-1950 they were likely associated with anthropogenic pressures such as increased human access and activity, particularly the construction of the Haast Pass Highway. In these cases, the second most significant assemblage shift was also reported. For visual purposes, taxa that showed marked changes either side of the assemblage zone were included in the plot.

Generalized Additive Mixed Model

Principal response curves were calculated for each taxonomic group based on Bray Curtis dissimilarity matrices. The function 'prcurve' from the package 'analogue' v0.17-6 (Simpson and Oksanen 2021) was used to calculate principal response curves for each depth downcore, using correspondence analysis to initialize the curve. Principal response curve scores were then plotted against Age and a Generalised Additive Mixed Model (GAMM) was fitted with an autocorrelation structure of order 1 (CorCAR1) to account for temporal autocorrelation using the 'mgcv' package v1.9-0 (Wood 2011). Due to the non-linearity of GAMMs, the method of finite differences was used to estimate derivatives of the spline to determine significant points of change in the model. Confidence intervals for the first derivative of the function were then calculated and plotted using the 'gratia' package (Simpson 2023), allowing for significant periods of change to be identified.

Results

Fish community and brown trout introduction

Using the 12S rRNA gene (Fish12S), nine fish taxa were found throughout the sediment core with fish reads averaging 313 reads per sample (± 113 SE). Seven of these taxa were identified to species level, six of which were

native New Zealand fish (*Galaxias argenteus*, *Galaxias fasciatus*, *Retropinna retropinna*, *Anguilla australis*, *Anguilla dieffenbachii* and *Gobiomorphus breviceps*), while *Galaxias* sp. was only identifiable to genus level (Fig. 2a). *Salmo trutta* (brown trout) and *Oncorhynchus tshawytscha* (Chinook salmon) were also identified, appearing in 1933 (1923–1943 CE with 95% high probability density function range) and 1964 (1961–1967 CE) based on this gene, respectively.

Using the Fish18S dataset, four ASVs from the genus *Galaxias* and one ASV from the *Salmo* genus were recovered, while five other ASVs were only classified to family (Gobiiformes) or class (Actinopteri). In this dataset, an unclassified species of *Galaxias* was the most relatively abundant ASV throughout the sediment core, recovered in every sample at high read abundances (Fig. 2b). Given the only *Salmo* found in Lake Paringa is *S. trutta*, we used the earliest 18S rRNA detection as the first sedDNA confirmation of this species in the lake (1906; 1892–1919 CE). Detection of *S. trutta* using the 18S rRNA gene was relatively consistent thereafter (Fig. 2b).

Differences in the native fish community were evident pre and post *S. trutta* detection, with the Fish12S data revealing *Galaxias argenteus* (giant kōkopu) as well as *G. fasciatus* (banded kōkopu) appearing only during the pre-trout phase (Fig. 2a). Salmon DNA (*Oncorhynchus* sp.) was only recovered in one sample from 1964 (1961–1967 CE), while *S. trutta* was recovered in five samples from 1933 (1923–1943 CE) to 1954 (1950–1958 CE).
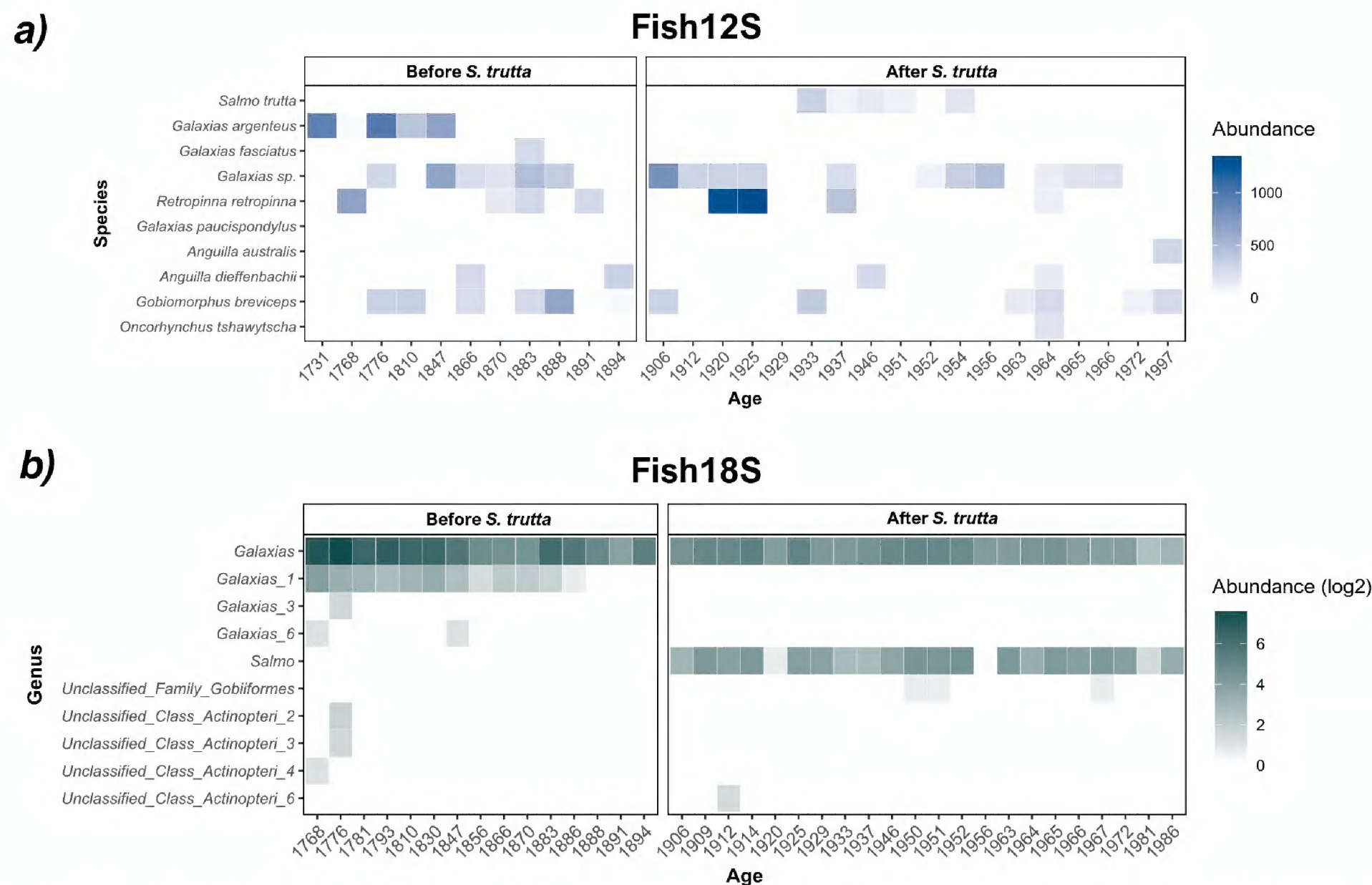
Figure 2. Heatmaps of fish taxa found in the Lake Paringa sediment core before and after the introduction of *Salmo trutta* (brown trout). Amplicon sequence variants (ASVs) taxonomically assigned to species level using the 12S rRNA primers are shown in (**a**), and individual ASVs from the 18S rRNA primers are shown in (**b**).
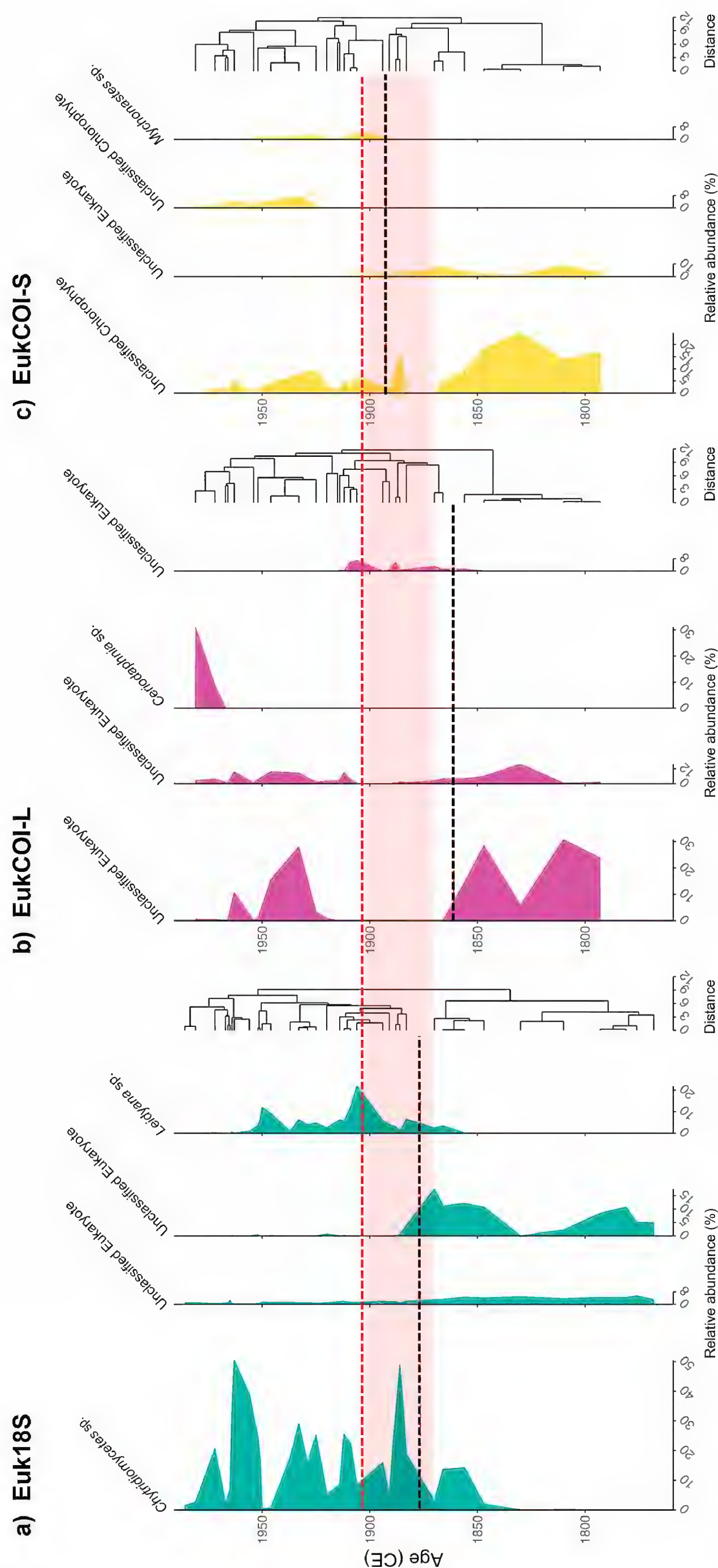
Eukaryotes

After rarefaction and removal of terrestrial taxa there were on average 11,780 reads per sample and a total of 3,123 ASVs for the Euk18S dataset. The EukCOI-L dataset had an average of 10,847 reads and 3,948 ASVs, while the EukCOI-S dataset had an average of 3,813 reads and 7,521 ASVs. The Euk18S community was dominated by Fungi and Apicomplexa, with a large proportion of unclassified taxa (Suppl. material 1: fig. S3a). Both EukCOI datasets were dominated by Annelida, Arthropoda and Chlorophyta, while the EukCOI-L community also had high proportions of Fungi (Suppl. material 1: fig. S3b, c). The CONISS analysis of the eukaryotic communities retrieved using all three primer sets indicates the main shift in community structure occurred between 1863 (1852–1881 CE) and 1876 (1857–1884 CE; Fig. 3). In the Euk18S dataset, this shift was driven largely by unclassified eukaryotic taxa, an increase in chytrid fungi and shifts within the alveolate (*Leidyana* sp.) community. In the COI datasets (EukCOI-L and EukCOI-S), this zonation is related to shifts in unclassified eukaryotes and green algae (unclassified chlorophytes and *Mychonastes* sp.). GAMM analysis of both the Euk18S and EukCOI-S datasets corroborated the CONISS, with significant periods of change encompassing the CONISS-indicated shifts found for each gene (1876 [1857–1884 CE] and 1863 [1852–1881 CE] respectively; Suppl. material 1: fig. S4). However, GAMM analysis of the Euk18S dataset suggests that eukaryotic community composition was undergoing significant change over a much broader time period from 1810 (1793–1826 CE) to 1920 (1908–1933 CE; Suppl. material 1: fig. S4a), while no significant period of change was signalled from the GAMM analysis for the EukCOI-L (Suppl. material 1: fig. S4b, c).

Prey invertebrates

When subsetting the eukaryotic data into phyla likely to be consumed by trout (phyla: Arthropoda, Mollusca and Rotifera), there were on average 712 reads per sample and 29 ASVs for the Prey18S dataset. The PreyCOI-L dataset had an average of 401 reads per sample and a total of 536 ASVs while for the Prey-COI-S dataset there were an average of 567 reads per sample and a total of 869 ASVs. The majority of reads in these subsets were unclassifiable beyond order, particularly for the COI markers (51–63% unclassified) while mites (e.g., *Maculobates* sp., Sarcoptiformes and Trombidiformes) dominated the Prey18S dataset (Suppl. material 1: fig. S5). A *Ceriodaphnia* sp. appears in PreyCOI-L in the late 1960's and increases to heavily dominate the group (~90% of reads in 1981 [1976–1987 CE]); however, this taxon was not identified using either the Prey18S or PreyCOI-S primers.

The main significant shift in these assemblages occurred between 1964 (1961–1967 CE) and 1972 (1971–1974 CE) in the Prey18S and PreyCOI-L datasets, and 1878 (1871–1895 CE). The second most important shifts occurred around 1891 (1877–1904 CE) and 1929 (1918–1940 CE) for the Prey18S and PreyCOI-L markers, respectively (Fig. 4). The more recent shifts (post-1965) appeared driven by the presence and significant increase in *Ceriodaphnia* sp. based on the PreyCOI-L. Shifts occurring pre-1900 appeared driven by an unclassified Spirostreptida (millipede) and unclassified Arthropods in the Prey18S and PreyCOI-S markers, respectively. The Prey18S community shift around 1891 (1877–1904 CE) was corroborated by a GAMM which found a significant

Figure 3. Statigraphic plot of eukaryotic sequences retrieved from (**a**) 18S rRNA (Euk18S), (**b**) long COI (EukCOI-L), and (**c**) short COI (EukCOI-S) primers across ~200 yrs of Lake Paringa sediment. CONISS derived dendrograms and assemblage change points (black dashed lines) are shown alongside selected taxa. The red dashed line indicates when *Salmo trutta* DNA appeared in the core (1906 [1892–1919 CE]) while the pale red box indicates the period *S. trutta* are expected to have been introduced (1870–1906 [1892–1919 CE]).
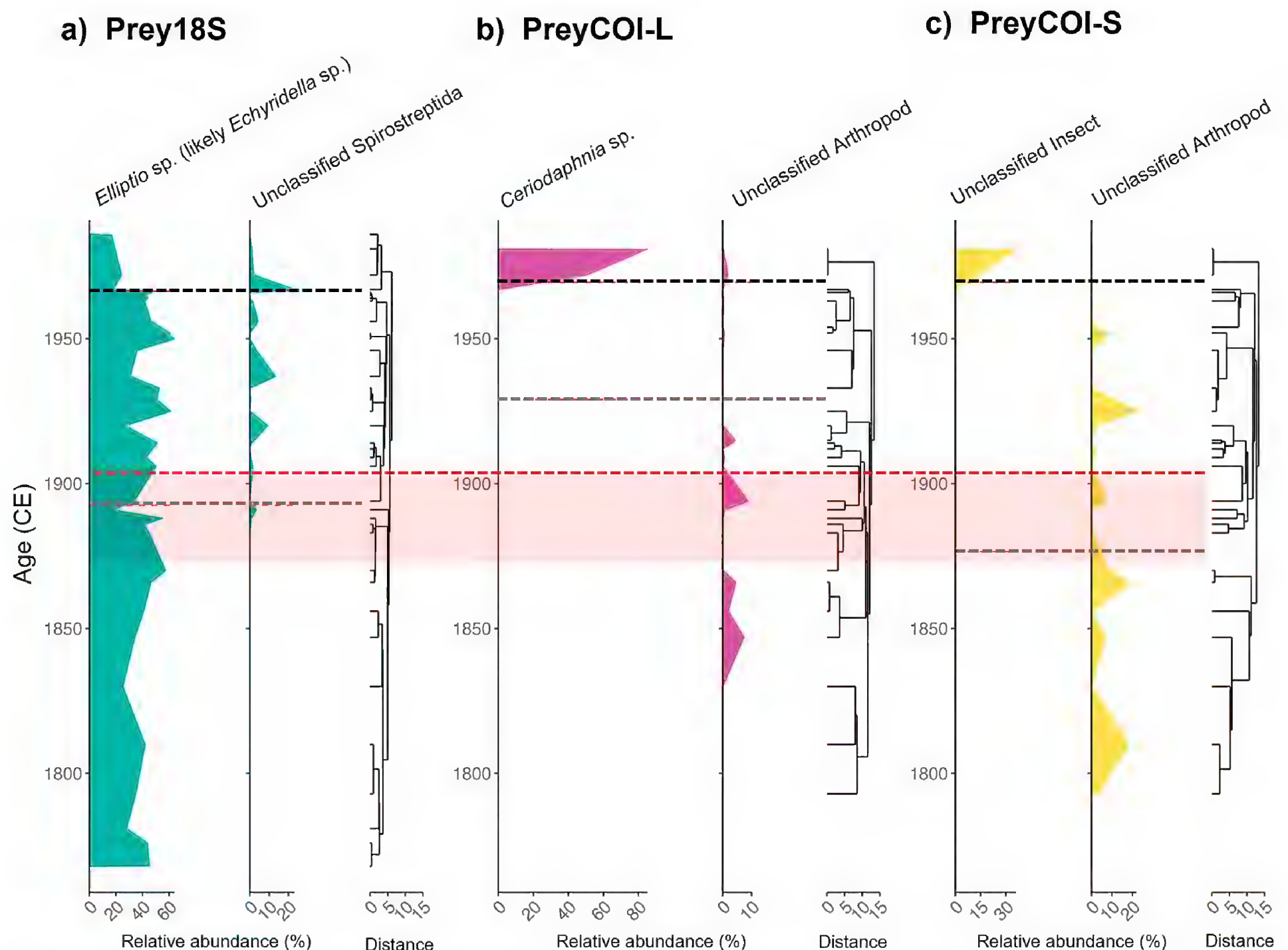
Figure 4. Statigraphic plot of taxonomic groups commonly eaten by *Salmo trutta* in Aotearoa-New Zealand (Arthropoda, Mollusca and Rotifera). Sequences were retrieved from: (**a**) 18S rRNA (Prey18S), (**b**) long COI (PreyCOI-L), and (**c**) short COI (PreyCOI-S) primers over ~200 yrs in Lake Paringa sediment. CONISS derived dendrograms and assemblage change points (black dashed lines = primary, grey dashed lines = secondary) are shown alongside selected taxa. The red dashed line indicates when *S. trutta* DNA appeared in the core (1906 [1892–1919 CE]) while the pale red box indicates the period *S. trutta* are expected to have been introduced (1870–1906 [1892–1919 CE]).

period of change between around 1847 (1831–1863 CE) and 1909 (1985–1922 CE). The assemblage shift in 1929 (1918–1940 CE) using PreyCOI-L was corroborated by a GAMM (Suppl. material 1: fig. S6).

Algal community

Algae recovered from the Algae18S dataset had an average of 45 reads per sample and 40 ASVs. AlgaeCOI-L had an average of 274 reads per sample and 83 ASVs, while AlgaeCOI-S had on average 1,166 reads per sample and 142 ASVs in total. The algal communities found using all primer sets were heavily dominated by green algae (unclassified Chlorophyta, *Choricystis* sp., and *Mychonastes* sp.; Suppl. material 1: fig. S7). CONISS analysis suggested the major primary shift in algal community assemblage across the studied time period was around 1870 (1857–1884 CE), 1929 (1918–1940 CE) and 1946 (1940–1951 CE) according to the Algae18S, AlgaeCOI-L and AlgaeCOI-S markers, respectively (Fig. 5). Secondary shifts occurred in 1953 (1948–1956 CE) and 1883 (1871–1895 CE) using the AlgaeCOI-L and AlgaeCOI-S datasets, respectively.
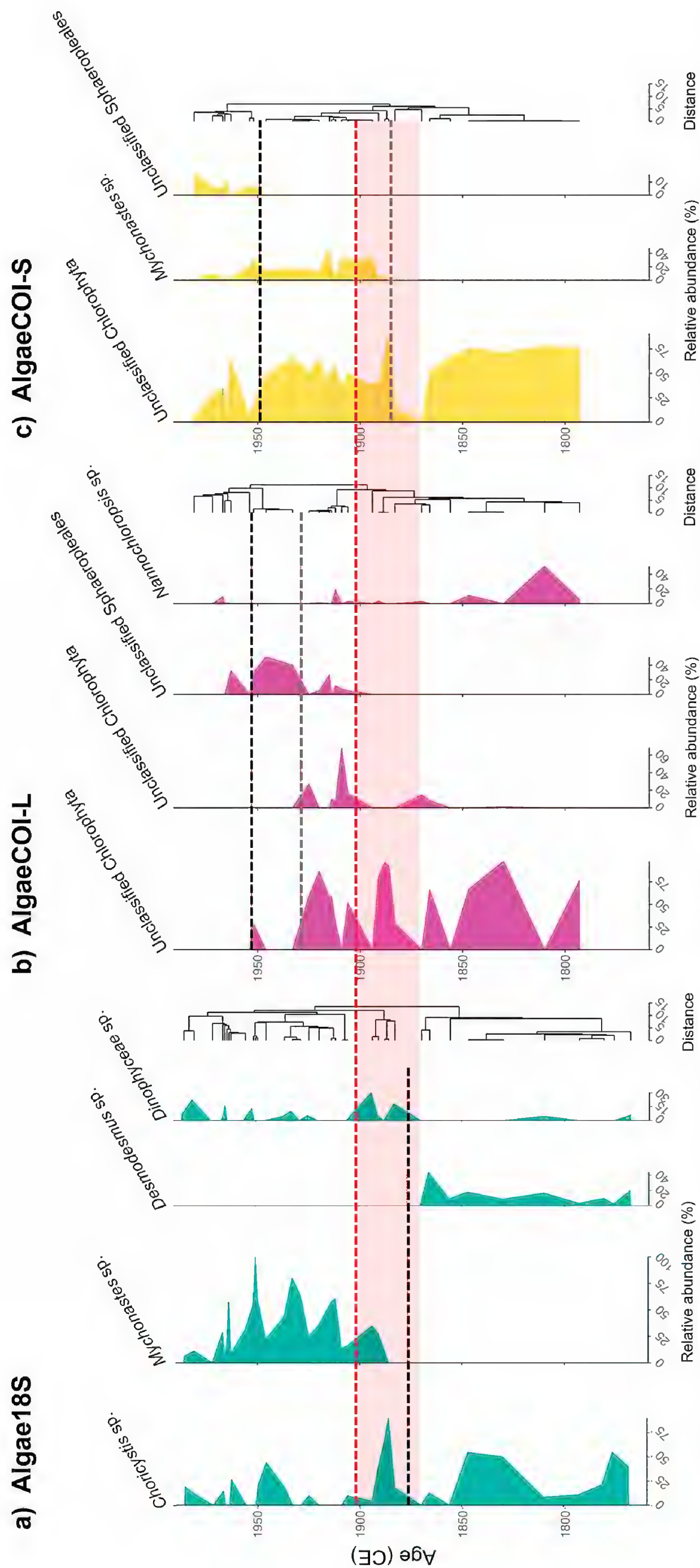
Figure 5. Statigraphic plot of Algal amplicon sequence variants retrieved from (**a**) 18S rRNA (Algae18S), (**b**) long COI (AlgaeCOI-L), and (**c**) short COI (AlgaeCOI-S) primers over ~200 yrs in Lake Paringa sediment. CONISS derived dendrograms and assemblage change points (black dashed lines = primary, grey dashed lines = secondary) are shown alongside selected taxa. The red dashed line indicates when *S. trutta* DNA appeared in the core (1906 [1892–1919 CE]) while the pale red box indicates the period *S. trutta* are expected to have been introduced (1870–1906 [1892–1919 CE]).

The shift occurring between 1870 (1857–1884 CE) and 1883 (1871–1895 CE) reflected in both the Algae18S and AlgaeCOI-S datasets was driven by a large increase in a *Mychonastes* sp. around 1886 (1874–1899 CE) when it increased up to 90% and 50% of the algal community, respectively (Fig. 5). *Choricystis* sp. (Algae18S) and Unclassified Chlorophyte (AlgaeCOI-S) ASVs also appear related to this shift. Later shifts from 1929 (1918–1940 CE) to 1946 (1940–1951 CE) captured by the AlgaeCOI-L and AlgaeCOI-S markers were driven by *Sphaeropleales* sp. and the decline of unclassified Chlorophyte ASVs (Fig. 5). GAMM's applied to the Algae18S dataset suggest a significant period of change between 1883 (1871–1895 CE) and 1906 (1892–1919 CE), which given the 95% high density probability function for the age model at this time may include the CONISS delimiter around 1870 (1857–1884 CE). However, no significant periods of change were found when applying GAMMs to either of the COI datasets (Suppl. material 1: fig. S8).

Total pigments

The total pigment flux was highest (> 1.5 µg cm^{-2} y^{-1}) in 1774 CE (1754–1794 CE), 1821 (1798–1844 CE), and 1970 (1968–1971 CE; Suppl. material 1: fig. S9a). The concentration of pigments was relatively low throughout the study period, with the highest concentration being 4.16 µg/g in 1774 (1754–1794 CE; Suppl. material 1: fig. S9b). A t-test showed no significant difference in total pigment flux before and after *S. trutta* arrival (p = 0.7, Suppl. material 1: fig. S9a).

Discussion

Potential foodweb effects

Fish community

Salmo trutta was observed using the Fish18S marker, with sequences attributed to a *Salmo* sp. occurring around 1906 (1892–1919 CE), and consistent recovery thereafter. We presume that trout introduction occurred sometime after 1870 CE and speculate that biomass was insufficient for it to be detected using the molecular approach until 1906 (1892–1919 CE), as previous studies have shown that DNA detection increases with higher biomass (Nelson-Chorney et al. 2019; Kuwae et al. 2020). Unfortunately, there are no written records to confirm the exact timing of trout introduction in Lake Paringa.

Changes in the historical fish community of Lake Paringa were evident using both 18S (Fish18S) and 12S rRNA (Fish12S) markers, with a shift from a galaxiid to *S. trutta* dominated community after about 1906 (1892–1919 CE). Salmonids such as *S. trutta* have been shown to negatively impact native fish populations across Aotearoa-New Zealand, proving particularly detrimental to galaxiid species which historically dominated many freshwater ecosystems (Rowe et al. 2002; McDowall 2006). Both markers reveal a pre-trout native fish community dominated by *Galaxia* sp. including *G. argenteus* (giant kōkopu) and *G. fasciatus* (banded kōkopu) which declined in presence and relative abundance just prior to the detection of *S. trutta* DNA (around 1906 [1892–1919 CE]) and disappear thereafter. Similar declines have been recorded in streams and rivers throughout Aotearoa-New Zealand (Crowl et al. 1992; McDowall 2006), where only one of 30 known galaxiid species is currently classified as 'not threatened' (Dunn et al. 2017).

Studies have shown that both predation and competition for food and habitat by introduced Salmonids, in particular *S. trutta*, have led to disjunct distributions and dwindling numbers of Galaxiids in streams and rivers across Aotearoa-New Zealand (Taylor and Main 1987; Minns 1990; Bonnett and Sykes 2002; David et al. 2002; McIntosh et al. 2010). In the present study, the sedDNA suggests this may also be the case for lake populations, which has been highlighted by McDowall (2006). However, the often-patchy recovery of fish sedDNA (Capo et al. 2021), such as was found for *G. fasciatus*, *A. australis* and *Onchorhynchus tshawytscha* using the 12S rRNA marker, indicates that more replication (e.g. cores, DNA extraction, PCR), larger sample sizes, and sediment cores taken from locations where fish are abundant (i.e. near the shore; Thomson-Laing et al. 2023) may be required to obtain greater quantities of fish sedDNA, in order to be confident results are not skewed by false negatives.

The introduction of new apex fish predators is often associated with foodweb trophic cascades through the creation and release of predation pressures at differing levels of the foodweb (Carpenter et al. 2001; Tronstad et al. 2010; Strock et al. 2013; Milardi et al. 2016). However, specific cascading effects are dependent on the number of pre-existing trophic levels occupied e.g., whether previous apex predators were piscivorous or planktivorous (Su et al. 2021). Another potential response is that in which a newly introduced predator in part or wholly replaces pre-existing predators with no resulting cascading effects. In Aotearoa-New Zealand, trout are known to displace native generalist fish taxa such as kōaro and kōkopu (McIntosh et al. 2010), therefore the lack of cascading effects on smaller, planktivorous fish (e.g., bullies, smelt and other galaxiids) may in part be due to *S. trutta* replacing the pre-existing generalist/piscivorous controls on these species.

Post 1906 (1892–1919 CE), a signal for *S. trutta* was consistently observed in the sediment core using the Fish18S marker. *Salmo trutta* sequences were also recovered using the Fish12S marker, although these were only found in < 20% of samples post-introduction. A comparison of these two markers shows potential similarities in fish communities; however, precise comparisons are difficult due to the disparity in taxonomic resolution between the two genes. While sequences recovered using Fish18S were only identifiable to class, family, and genus level, the Fish12S data could be assigned to species level using a manually constructed database of known 12S rRNA fish sequences. There is agreement between the markers at the class, family, and some cases genus level, for example, sequences from both markers include *Galaxias* and *Salmo* genera as well as the Gobiiformes family. The lack of taxonomic discrimination using the 18S rRNA gene could be due to this region of the gene being too conserved for fish species and/or a lack of fish sequences in the 18S rRNA database. Consequently, while this gene was successful in recovering fish DNA more consistently, particularly for *S. trutta*, the lack of taxonomic resolution is limiting. In the present study, the use of multiple markers proved to be complementary. We were able to capitalise on the taxonomic resolution of the 12S marker while benefiting from the more consistent recovery of fish sequences using the 18S marker. However, given that the species-specificity of metabarcoding assays can be low, another approach to identify the timing of *S. trutta* introduction would be the use of a quantitative, species-specific assay. A targeted *S. trutta* assay using qPCR or ddPCR could help improve detection probability, potentially providing a more accurate estimate of the timing of *S. trutta* introduction.

Lower foodweb effects

The cascading effects of trout introduction are often visible in aquatic ecosystems via shifts in zooplankton and algal dynamics or community structure, and even changes in zooplankton body-size distributions (Tronstad et al. 2010; Strock et al. 2013; Milardi et al. 2016). The most significant eukaryotic community shift across the ~200-year period from all markers was between 1863 (1852–1881 CE) and 1876 (1857–1888 CE; Fig. 3), encompassing the probable timing of *S. trutta* introduction. Interestingly, this was related to different taxa in the two different genes analysed. Using the two COI markers, green algae (Chlorophyta and *Mychonastes* sp.) appeared to drive the community shifts, while unclassified Eukaryotes and shifts in the relative abundances of different Alveolates (*Leidyana* sp.) appeared responsible for the shift detected using the 18S rRNA marker. Chytrid fungi (Chytridiomycetes) heavily dominated the Euk18S dataset, increasing in relative abundance from around 1830 (1814–1847 CE), coinciding with European settlement of Aotearoa-New Zealand. Some Chytrid fungi are highly parasitic to phytoplankton species (Kagami et al. 2007), which could explain the decline in two *Choricystis* sp. between around 1830 (1814–1847 CE) and 1883 (1871–1895 CE) and/or a *Desmodesmus* sp. which disappears in 1866 (1852–1881 CE). Due to a lack of sequences in the databases, other taxa related to this shift were largely unclassified Eukaryotes and unclassified Chlorophytes.

Lake Paringa was chosen for this study due to its relatively unmodified catchment which is almost entirely indigenous forest. While much of the country was experiencing deforestation and the rapid growth of pastoral agriculture during the time of European settlement (mid to late 1800s), the Lake Paringa catchment remained forested. European settlers began to access the surrounding area around 1875 when a small cattle track was constructed to the east of the lake, allowing small herds of cattle to be moved north. Minor amounts of nutrients may have been deposited near, or in, the lake during the movement of cattle, although the impact of these sporadic cattle-drives is likely to be low. Similarly, the introduction of wild terrestrial mammals including red deer and marsupials such as possums to the region in the mid to late 1800s may have released slightly more nutrients into the surrounding land with potential to enter the lake, but again, the in-lake effects are likely to be very minor. Algal pigment analysis corroborates the lack of productivity around that time, with only a small spike in 1850. The most major change in catchment land use would have been in 1965 when the Haast Pass Highway was constructed along the south-east side of the lake. Earthworks would have occurred and subsequent increases in vehicle traffic and visitors to the area may have impacted the lake, however this occurred much later than the introduction of *S. trutta*, which is the time of interest in this study. Therefore, there is a high likelihood that this significant shift in the overall eukaryotic community is related to the introduction of trout, given that no other major disturbances occur at this time that could explain the change in this broad range of taxa.

Trout diet studies reveal a broad range of organisms are consumed by these generalists including fish, aquatic and terrestrial arthropods, molluscs and rotifers (Rutledge 1991; Sagar and Glova 1995; Olsson et al. 2006). While changes in terrestrial arthropod communities may be related to terrestrial based processes such as the introduction of land mammals, they were included in this study due to the possibility that trout could reduce their sedDNA signal in the

lake samples, but primarily because the majority of arthropod sequences were unclassifiable. This makes it difficult to determine which of the unclassified taxa are terrestrial, or not part of the trout diet. As such, all arthropods were included in the dataset and their habitat noted where known. It should therefore be noted that the changes in arthropod signals over time cannot be directly associated with specific trout interactions.

When exploring our data on eukaryotic groups likely to be predated by trout (Arthropods, Molluscs and Rotifers), CONISS analyses identified the primary assemblage shift in two markers occurred post-1950 (1946–1953 CE), likely related to anthropogenic changes including increased human access. Primary and secondary shifts also occurred between 1878 (1871–1895 CE) and 1891 (1877–1904 CE), for the PreyCOI-S and Prey18S dataset respectively coinciding with the probable timing of *S. trutta* introduction. GAMM analysis corroborated this significant period of change in the Prey18S dataset but not using either PreyCOI datasets. The lack of detection of a significant period of change in the PreyCOI subsets could be due to the inconsistent and patchy recovery of many arthropod, mollusc and rotifer taxa making temporal changes difficult to assess. Most sequences in these datasets were unable to be taxonomically classified, impeding interpretations on how trout are possibly affecting the lake community.

Different prey taxa were also found to be dominant depending on the marker used. For example, freshwater mussels (classified as *Elliptio* sp. but likely to be *Echyridella* sp.) dominated the Prey18S dataset along with a range of mites classified to genus and sometimes order level. Whereas, no specific ASVs dominated either COI datasets until 1967 (1964–1968 CE) when a *Ceriodaphnia* sp. appeared and increased dramatically in the PreyCOI-L dataset. The recovery of ASVs was patchy using both COI markers where many taxa were found only a few times throughout the core, making time-series analysis of these results difficult.

Of note are the lack of known Aotearoa-New Zealand zooplankton (Rotifers, Copepods and Cladocera) retrieved throughout the sediment core which are consistently lacking across all three markers except for the significant increase in a *Ceriodaphnia* sp. in 1967 (1964–1986 CE) using the EukCOI-L marker. This was unexpected for the two COI markers, given that in this study both the EukCOI-L forward and EukCOI-S reverse primers were specifically adapted to increase base-pair matches with a range of Aotearoa-New Zealand zooplankton species. In addition, the 18S primer has successfully retrieved zooplankton reads from contemporary water samples in a previous study (Pearman et al. 2022) indicating this may be a methodological challenge relating to DNA preservation or recovery of these organism from lake sediments. Consequently, any potential zooplankton effect could not be determined using this sedDNA approach. As zooplankton are known to be components of trout diets, the poor retrieval of these species in the DNA could potentially result in shifts in these vital components of the food web being missed and further work to improve the detection of zooplankton in lake sediment eDNA studies is required.

There was evidence of a significant shift in algal taxa around the time of trout introduction. The Algae18S marker suggests this as the most major assemblage shift, while the AlgaeCOI-S marker found this was the second most major shift. Shifts in diatom species assemblages have been recorded following trout introduction (Sienkiewicz and Gąsiorowski 2016; Cantonati et al. 2021). However, in this study, a general decrease in certain species of green algae alongside a

significant increase in *Mychonastes* sp. and other unclassified algal species appear responsible for the assemblage shift occurring after the introduction of *S. trutta*. The sudden presence and subsequent increase in *Mychonastes* sp. found by both Algae18S and AlgaeCOI-S markers, and potentially by the AlgaeCOI-L marker as an ASV of the order Sphaeropleales, appear to have driven the significant algal assemblage shift between 1870 (1857−1884 CE; Algae18S) and 1883 (1871−1895 CE). The appearance and growth of the small (< 3 μm), coccoid, eukaryotic picoplankton *Mychonastes* sp. (Liu et al. 2020) could suggest a relaxation of grazing pressure at the micro- or macro-zooplankton level, following the introduction of *S. trutta*. However, these impacts are unable to be directly determined due to the lack of zooplankton recovered throughout the core.

The shifts in algal community structure observed in Lake Paringa did not correspond with a shift in algal pigment concentrations (as an indication of primary productivity in the lake). There were no significant changes in total pigment flux associated with this shift in algal community structure and patterns in algal pigments were consistent pre and post introduction of *S. trutta*. This is different from observations in other studies (Tronstad et al. 2010; Strock et al. 2013), although those algal shifts recorded as a result of trophic cascades may be due to a corresponding increase in nutrient levels in those systems. Alternatively, the lack of obvious pigment shifts could also be due to the low pre-existing algal abundance in this oligotrophic lake.

Sedimentary DNA challenges and recommendations

Overall, sedDNA results suggest changes to the Lake Paringa fish, eukaryotic and algal communities occurred around 1856 (1841−1871 CE) to 1891 (1877−1904 CE), which could be related to the timing of *S. trutta* introduction to the lake, which was first detected in the sedDNA in 1906 (1892−1919 CE). The metabarcoding approach identified possible responses from a wide range of organisms, including microscopic (e.g., fungi) and soft-bodied (e.g., polychaetes) taxa which would not be detectable using traditional paleolimnological approaches. However, there are significant caveats to the use and interpretation of sedDNA that need to be considered.

In this study, the data presented are from a single core location from one lake and are therefore limited in their potential extrapolation to other lakes. Collecting cores from multiple unimpacted lakes with introduced trout would add evidence to the pattern observed here. To explore shifts in foodweb components, we used a multiple markers approach to reduce the chance that key taxa were missed due to primer biases (Zhang et al. 2018). However, we still failed to robustly detect some taxonomic groups of interest (e.g., zooplankton). The 18S rRNA primers (Zhan et al. (2013) have been successful in previously detecting zooplankton species in Aotearoa New Zealand both in water and lake surface sediments (Pearman et al. 2022), however very few sequences were recovered in this study. Although three different markers were used, zooplankton taxa reads were absent or very low throughout the majority of the sediment core. While two of the three primers were adapted to increase recovery of zooplankton found in Aotearoa-New Zealand, further *in vitro* analysis needs to be undertaken to determine why these taxa were not amplified. This could be achieved through sediment-spiking, in which zooplankton remains are added

to samples of core sediment before extracting DNA in order to assess DNA recovery (Brasell et al. 2022). It is possible that lake-specific sediment chemistry may negatively impact DNA recovery of certain organisms, and this should be further investigated. It should also be noted that for the algal subset of the 18S rRNA data, very low read numbers were obtained. This could be due to the universal nature of the primer targeting other eukaryotes.

The patchy recovery of certain taxa such as fish is also of concern. A number of species likely to have inhabited the lake throughout the study period were only found sporadically. For example, *Anguilla australis* and *A. dieffenbachii*, two native eels known to inhabit freshwaters of the West Coast region prior to 1700 CE, and which still exist today, are recovered in only five samples collectively. Inconsistent recovery of DNA from larger, motile organisms such as fish is a challenge in lake sediments (Thomson-Laing et al. 2022), as DNA can be heterogeneously distributed throughout lakes. However, taxa recovered using both COI primers were noticeably patchy in their recovery, meaning the community shifts calculated for those data subsets were largely driven by changes in rare and / or inconsistently recovered taxa. This could be due to the COI primers being designed to target multicellular organisms which have a more patchy distribution in sediment compared to microbial species which are also amplified with the 18S rRNA primers. Variability in DNA recovery could also be related to changes in the sedimentology, and further study is needed to explore this in detail.

While work is ongoing to populate databases with the huge influx of newly discovered sequences, these databases are still lacking in many areas, particularly those of smaller, less charismatic organisms. The overwhelming majority of ASVs recovered from this sediment core were unclassifiable below order level, illustrating the lack of Aotearoa-New Zealand species represented in major databases. While information is still contained in this data, validation and ground-truthing requires taxonomic classification, which was unobtainable for the majority of sequences. Hence the pressing need to incorporate species from diverse geographic areas such as Aotearoa-New Zealand into sequence databases.

Conclusion

In this study, we used a multi-marker sedDNA approach to investigate changes across multiple trophic levels that correspond with the timing of *S. trutta* introduction to Lake Paringa. Using multiple DNA markers, convincing community changes were revealed throughout the foodweb, indicating that the introduction of *S. trutta* can substantially affect the ecology of lakes. Using sedDNA we were able to successfully detect organisms that were unidentifiable using traditional paleolimnological techniques, so providing a more holistic assessment of the temporal effects of *S. trutta* introductions on lake organisms. However, limitations with the sedDNA method meant that many of the taxa responsible for assemblage shifts could not be identified to genus or species level. Therefore, the ecological mechanisms driving these shifts, i.e., top-down pressure from *S. trutta*, were unable to be identified or confidently linked to their introduction. Pigment analysis indicated no major shift in algal concentration associated with the introduction of *S. trutta*, suggesting that typical cascading effects linked to salmonid introduction may not have occurred in this lake. Challenges with consistent DNA recovery due to the patchy distribution of some organisms

and the nature of historic lake sediments identifies the need for sampling optimisation (including amount of sediment analysed and further sample replication), extraction optimisation and primer validation for taxa of interest.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: DK, SAW, MJV. Data curation: JKP, LAS. Formal analysis: LAS, GTL, AR, JP. Funding acquisition: MJV, SAW. Methodology: JDDH. Writing - original draft: LAS. Writing - review and editing: JDDH, GTL, JKP, DK, JP, SAW, MJV, SF, AR.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary data 1

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Data type: pdf

Explanation note: **table S1.** Core subsamples used for sedimentary DNA analysis and corresponding dates from the age model. **table S2.** PCR cycling conditions for the primers used in this study. **fig. S1.** Sediment core images. Red boxes show location of samples as described in table S1. **fig. S2.** Rarefaction curves before and after rarefying for the 18S rRNA gene (Euk18S), short COI gene (EukCOI-S), long COI gene (EukCOI-L) and 12S rRNA gene (12S). **fig. S3.** Proportional abundances of eukaryotic phyla retrieved from three different primer sets; (a) 18S rRNA (Euk18S), (b) long COI (EukCOI-L), and (c) short COI (EukCOI-S). **fig. S4.** Principal response curve scores of eukaryote community structure through the sediment core. **fig. S5.** Proportional abundances of Arthropod, Mollusc and Rotifer orders retrieved using three different primer sets (a) 18S rRNA (Prey18S), (b) long COI (PreyCOI-L), and (c) short COI (PreyCOI-S). **fig. S6.** Principal response curve scores of taxa common in the diet of *Salmo trutta*. **fig. S7.** Proportional abundances of algal classes retrieved from; (a) 18S rRNA (Algae18S), (b) long COI (AlgaeCOI-L), and (c) short COI (AlgaeCOI-S). **fig. S8.** Principal response curve plots of algal community structure through ~200 yrs of Lake Paringa sediment core. **fig. S9.** Sediment-derived total pigment flux (a) and total pigment concentration (b) from 1774 to 2019 determined using high performance liquid chromatography.

Link: https://doi.org/10.3897/mbmg.7.111467.suppl1

Supplementary material 2

Supplementary data 2

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Data type: xlsx

Explanation note: **table S3.** Algae 18S. **table S4.** Algae COI-L. **table S5.** Algae COI-S. **table S6.** Euk 18S. **table S7.** Euk COI-L. **table S8.** Euk COI-S. **table S9.** Prey 18S. **table S10.** Prey COI-L. **table S11.** Prey COI-S.

Link: https://doi.org/10.3897/mbmg.7.111467.suppl2